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**Srivastava**(10) **Patent No.:** **US 6,261,834 B1**  
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(21) **Appl. No.:** **07/982,193**(22) **Filed:** **Nov. 25, 1992****Related U.S. Application Data**(63) **Continuation-in-part of application No. 07/789,917, filed on Nov. 8, 1991, now Pat. No. 5,252,479.**(30) **Foreign Application Priority Data**

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(51) **Int. Cl.<sup>7</sup>** ..... **C12N 15/861; C12N 5/16**(52) **U.S. Cl.** ..... **435/320.1; 435/325; 435/372**(58) **Field of Search** ..... **435/235.1, 240.2, 435/320.1, 325, 372, 375, 456, 462; 536/23.1, 24.1, 24.5**(56) **References Cited****U.S. PATENT DOCUMENTS**5,139,941 8/1992 Muzyczka et al. .  
5,173,414 12/1992 Lebkowski et al. .  
5,252,479 \* 10/1993 Srivastava ..... 435/235.1**FOREIGN PATENT DOCUMENTS**

WO88/08450 11/1988 (WO) .

**OTHER PUBLICATIONS**Izban et al. (1989), *J. Biol. Chem.* 264 (16): 9171-9179.\*  
Kim et al. (1985), *Cell* 42: 129-138.\*  
Lu et al. (1987), *J. Immunol.* 139(6): 1823-1829.\*  
Ohi et al. (1990) "Construction and Replication of an Adeno-Associated Virus Expression Vector that Contains Human $\beta$ -Globin", *Gene* 89, 279-282.  
Vincent et al. (1990) "Replication and Packaging of HIV Envelope Genes in a Novel Adeno-Associated Virus Vector System", *Vaccines* Cold Spring Harbor Laboratory, New York, 353-359.  
Blundell et al. (1987) "In Vitro Identification of a B19 Parvovirus Promoter", *Virology* 157, 534-538.  
Blundell et al. (1989) "A GC-Box Motif Upstream of the B19 Parvovirus Unique Promoter is Important for In Vitro Transcription", *J. Virol.* 63, 4814-4823.  
Doerig et al. (1987) "A Transcriptional Promoter of the Human Parvovirus B19 Active In Vitro and In Vivo", *Virology* 157, 539-542.  
Doerig et al. (1990) "Nonstructural Protein of Parvoviruses B19 and Minute Virus of Mice Controls Transcription", *J. Virol.* 64, 387-395.Hermonat et al. (1984) "Use of Adeno-Associated Virus as a Mammalian DNA Cloning Vector: Transduction of Neomycin Resistance into Mammalian Tissue Culture Cells", *Proc. Natl. Acad. Sci. USA* 81, 6466-6470.Kotin et al. (1990) "Site-Specific Integration by Adeno-Associated Virus", *Proc. Natl. Acad. Sci. USA* 87, 2211-2215.Lebkowski et al. (1988) "Adeno-Associated Virus: A Vector System for Efficient Introduction and Integration of DNA into a Variety of Mammalian Cell Types", *Mol. Cell. Biol.* 8, 3988-3996.Samulski et al. (1982) "Cloning of Adeno-Associated Virus into pBR322: Rescue of Intact Virus from the Recombinant Plasmid in Human Cells", *Proc. Natl. Acad. Sci. USA* 79, 2077-2081.Samulski et al. (1989) "Helper-Free Stocks of Recombinant Adeno-Associated Viruses: Normal Integration does not Require Viral Gene Expression", *J. Virol.* 63, 3822-3828.Shade et al. (1986) "Nucleotide Sequence and Genome Organization of Human Parvovirus B19 Isolated from the Serum of a Child During Aplastic Crisis", *J. Virol.* 58, 921-936.Srivastava et al. (1983) "Nucleotide Sequence and Organization of the Adeno-Associated Virus 2 Genome", *J. Virol.* 45, 555-564.Srivastava et al. (1989) "Construction of a Recombinant Human Parvovirus B19: Adeno-Associated Virus 2 (AAV) DNA Inverted Terminal Repeats are Functional in an AAV-B19 Hybrid Virus", *Proc. Natl. Acad. Sci. USA* 86, 8078-8082.McLaughlin et al. (1988) "Adeno-Associated Virus General Transduction Vector: Analysis of Proviral Structures", *Journal of Virology* 62, 1963-1973.Tratschin et al. (1984) "A Human Parvovirus, Adeno-Associated Virus, as a Eucaryotic Vector: Transient Expression and Encapsulation of the Prokaryotic Gene for Chloramphenicol Acetyltransferase", *Mol. and Cell. Biol.* 4, 2072-2081.

\* cited by examiner

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Gene therapy involves the transfer and stable insertion of new genetic information into cells. The present invention is directed to safe vectors for gene therapy and thus provides hybrid parvovirus vectors which are capable of site-specific integration into a mammalian chromosome without substantial cytotoxicity, and which direct erythroid cell-specific expression of heterologous genes. The hybrid vector is useful in gene therapy, particularly in the treatment of hemoglobinopathies and other hematopoietic diseases, and in conferring cell-specific multidrug resistance. A method of delivery of constitutive levels of a pharmaceutical product and a method of producing a recombinant protein are also provided.